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THE USE OF THE MOSQUITO BIOASSAY FOR DETERMINING THE TOXICITY TO MAN OF CIGUATERIC FISH

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Abstract

Moderately well-correlated with the mouse bioassay, requiring a much smaller amount of raw fish flesh, and employing a faster chemical extraction procedure, the mosquito bioassay represents significant progress in detecting ciguatoxin in fish muscle. We use the mosquito bioassay to evaluate the approximate amount of ciguatoxin present in the remains of 25 fish of 17 different species involved in 99 documented cases of ciguatera poisoning. From the data provided by mosquito bioassay and human symptomatology, we determine a dose-effect relationship for ciguatoxin (CTX) to man. Three average pathogenic doses (PD) are established. The minimum PD is estimated to be 0.6 ng CTX/kg, the PD50 to be 2 ng CTX/kg, and the PD100 to be 8 ng CTX/kg. These data suggest a MLD (minimal lethal dose) value of 20 ng CTX/kg.

Introduction

Ciguatera is the most frequently reported food-borne disease in French Polynesia. Its sporadic and scattered outbreaks, tied to natural and human phenomena, constitute a widespread and serious problem in these islands, both as a health hazard (Fig. 1) and as an obstacle to the exploitation of reef-fish resources (Fig. 2).

As accurate an evaluation as possible of ciguatera levels is imperative for use by local authorities. Until a simple, reliable, rapid chemical assay, like the one recently proposed by Hokama (1985), is available, bioassays will continue to be widely used. Usually, small animals are used for bioassays. Thus, the mosquito bioassay (Pompon *et al.,* 1984), which has a moderate correlation to the cat bioassay and the mouse bioassay (Bagnis *et al.,* 1985), represents significant progress in detecting ciguatoxin in fish muscle. The swiftness of the chemical extraction procedure (Pompon and Bagnis, 1984) and of the mosquito response, as well as the small amount of fish needed, allow us to check the ciguatoxicity of very small fish (Chungue *et al.,* 1984) or of the remains of poisonous meals. The following study aims to establish, from the data taken from the mosquito bioassay and human symptoms, a relationship between the approximate amount of ingested ciguatoxin (Scheuer *et al.,* 1967) and its clinical effects (Bagnis *et al.,* 1979).

Material and Methods

Patients

Patients either were seen in the consulting room of the Louis Malarde Medical Research Institute in Papeete or were hospitalized in the General Hospital in Papeete. Clinical symptoms were observed in all the poisoned people.

FIGURE 1. Geographical distribution of the incidence of ciguatera per 1000 inhabitants (C.I.R.) in French Polynesia (1960 to 1984).

Fish

Fish belonged to 17 species at several trophic levels and were caught in various areas of French Polynesia. Each fish sample was responsible for at least one documented case of ciguatera. The average amount of fish flesh eaten by an individual was evaluated by anamnesis and by weighing control rations.

Rapid extraction of the ciguatoxin

Ciguatoxin extraction procedures are detailed in Figures 1 and 2. A sample (8 g) of ground raw or cooked fish flesh was homogenized with 40 ml of acetone (twice) and centrifuged. The supernatants were concentrated and percolated through a cartridge of diatomaceous silica (4 g) that was washed successively by n-hexane (45 ml) and diethyl ether (45 ml). After evaporation, the diethyl ether toxic residue was subjected to fast, low-pressure chromatography on silicic acid (1 g). Elution was performed successively by chloroform (25 ml) and a chloroform-methanol mixture (9:1:25 ml). The resulting eluate was evaporated. From 8 g of fish flesh, 1.0 ± 0.2 mg of toxic residue was obtained within 1 h, regardless of the species or the toxicity of the fish.

Mosquito bioassay

The mosquitoes, weighing 1.6 ± 0.2 mg, belonged to the species *Aedes aegypti*. They were intrathoracically injected as described by Rosen and Gubler (1974). Each portion of extract obtained from 8 g of flesh was suspended in 100 μl of phosphate-buffered saline (pH 7.4, 0.002 *M*) containing 0.5% gelatin and 5% heated calf serum (56°C for 30 min). A volume of 0.5 μl of two-fold serial dilutions was injected into eight groups of ten mosquitoes each. The tested doses ranged from 0.31 to 40×10^{-3} g of flesh per mosquito. A control group received diluent. Mortality was observed 1

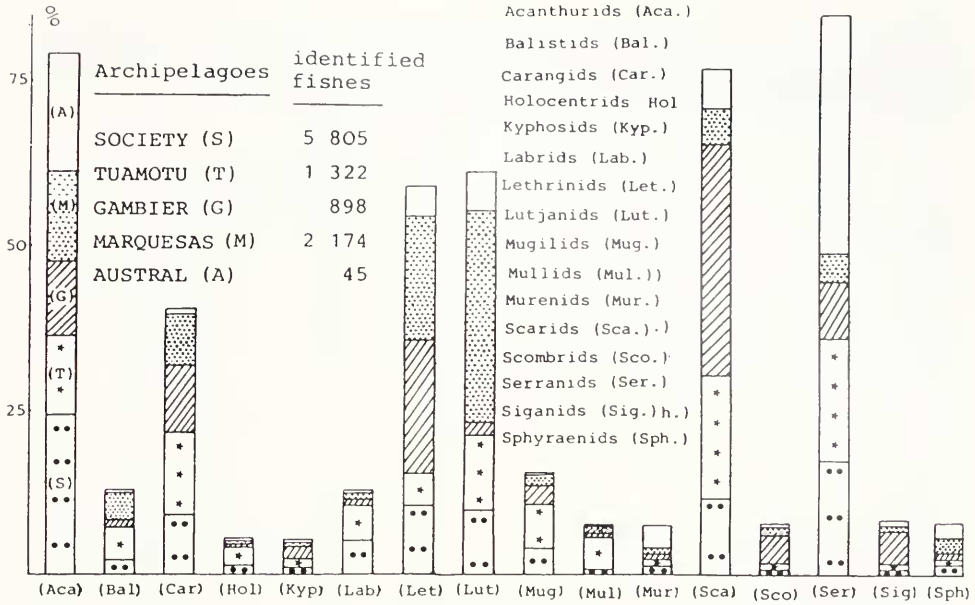

FIGURE 2. Geographical distribution of ciguateric fish in French Polynesia.

h after injection. The method of Bliss (1938) was used to calculate LD50 in mosquitoes. The fish ciguatoxicity was expressed in equivalent gram of fish (fge) or in *Aedes* mosquito-units (AU). A fish contains 1 AU of ciguatoxin when five mosquitoes in a group of ten die after each has received extract equivalent to 7 mg of fish.

Dose-effect relationship for ciguatoxin

Using as a baseline the MLD of pure ciguatoxin for mice [*i.e.*, 0.45 g/kg according to Tachibana (1980)], the correspondence between cat bioassay (Bagnis and Fevai, 1971), mouse bioassay (Yasumoto *et al.*, 1977), and mosquito bioassay have been previously established (Bagnis *et al.*, 1985). They are as follows:

$$1\ \text{AU} = 17.5 \times 10^{-5}\ \text{mouse-units (MU)} = 8.8 \times 10^{-5}\ \text{cat units (CU)}$$
$$= 1.6 \times 10^{-3}\ \text{ng CTX.}$$

RESULTS AND DISCUSSION

Epidemiological features

Twenty-five fish, eaten by 142 individuals, induced ciguatera symptoms in 99 people (see Table I). Among these, 30 were hospitalized for a period ranging from 24 hours to 10 days. The fish involved in the most severe cases were carnivorous fish from four species: *Lethrinus miniatus, Cheilinus undulatus, Aprion virescens,* and *Sphyraena barracuda.*

Clinical features

The most frequently observed symptoms are shown in Table II. Sensory disturbances were much more frequent than neuromotor symptoms.

TABLE I

Results of the mosquito bioassay for 25 fish involved in documented cases of ciguatera poisoning, with the corresponding toxicological data provided from the mouse bioassay and the cat bioassay

	Man		Mouse	Cat	Mosquito		
Fish	(a)	(b)	(c)	(d)	(e)	(f)	(g)
Lethrinus miniatus (Tautira)	6/8	3	0.23	0.12	1.02	6.9	1.54
Lethrinus miniatus (Raiatea)	8/10		1.00	0.18	2.70	2.6	0.58
Lethrinus miniatus (Vairao)	3/5		0.35	0.10	3.11	2.3	0.50
Lethrinus miniatus (Paea)	9/9	3	0.90	0.14	1.64	4.3	0.96
Cheilinus undulatus (Arue)	6/6	6	1.20	0.28	0.89	7.9	1.77
Cheilinus undulatus (Paea)	6/6	2	2.25	0.23	1.44	4.9	1.10
Cephalopholis argus (Tautira)	7/7	5	0.50	0.10	2.30	3.0	0.68
Cephalopholis argus (Teahupoo)	2/2		0.60	0.15	1.61	4.4	0.98
Epinephelus microdon (Mataiva)	4/7		0.18	0.11	2.05	3.4	0.77
Sphyraena barracuda (Moorea)	7/7		0.60	0.12	2.02	3.5	0.78
Aprion virescens (Marquesas)	6/6	6		0.14	0.83	8.4	1.90
Naso unicornis (Mataiva)	2/2		0.52	0.10	2.65	2.6	0.57
Scarus jonesi (Hao)	5/5		2.25	0.14	0.98	7.1	1.60
Scarus jonesi (Mataiva)	3/3		1.80	0.18	1.53	4.6	1.03
Lutjanus bohar (Cook)	3/3	3	0.29	0.18	2.37	3.0	0.67
Lutjanus bohar (Tetiaroa)	6/8		0.45	0.10	2.23	3.1	0.71
Monotaxis grandoculis (Mataiva)	2/2		0.36		1.45	4.8	1.09
Sphyraena barracuda (Rairoa)	5/5	2	0.74	0.10	2.22	3.3	0.74
Lutjanus gibbus (Papeete)	2/5		0.84		6.30	1.1	0.26
Caranx melampygus (Punaauia)	1/6		0.18	0.01	3.14	2.2	0.50
Plectropomus leopardus (Taenga)	1/8		0.09	0.10	4.77	1.5	0.33
Gymnosarda unicolor (Gambier)	2/5		0.30	0.02	6.19	1.1	0.26
Lutjanus gibbus (Papeete)	1/6		0.09		6.66	1.1	0.24
Crenimugil crenilabis (Mataiva)	1/5		0.15		7.95	0.9	0.19
Scarus gibbus (Hao)	1/5		0.10		7.11	1.0	0.22

LEGEND: (a) Number of patients/number of consumers.
(b) Number of hospitalized patients.
(c) Dose of ciguatoxin in nanograms per gram of fish (CTX:ng/fg) estimated by the mouse bioassay.
(d) CTX (ng/fg) estimated by the cat bioassay.
(e) LD50 in 10^{-3} fge per 1.6 mg mosquito.
(f) LD50 in mosquito-units (AU).
(g) CTX (ng:fg) estimated by the mosquito bioassay.

Of the 30 hospitalized patients, 24 displayed chiefly cardiovascular symptoms. Bradycardia with a pulse ranging from 40 to 60 beats/min (21% of cases), systolic blood pressure of 100 mm Hg or less (18% of cases), abnormalities of electrocardiograms (15% of cases) and cardiac distress (6% of cases) were observed. Twelve patients were hospitalized for other severe symptoms which may or may not have been associated with cardiovascular disturbances: ataxia (10), paresis (2), dyspnea (8), and dysuria (5).

TABLE II

Frequency of symptoms in 99 documented cases of ciguatera

Symptom	% of patients with finding*
1. Paresthesia of the extremities	92
2. Circumoral pareshesia	92
3. Burning or pain to skin on contact with cold water	91
4. Arthralgia	88
5. Myalgia	86
6. Diarrhea	74
7. Asthenia	66
8. Nausea	56
9. Pruritus	48
10. Headache	45
11. Abdominal pain	44
12. Chills	43
13. Vomiting	40
14. Bradycardia	38
15. Vertigo	33
16. Perspiration	32
17. Ataxia	28
18. Hypotension	28
19. Dental pain	24
20. Tremor	18
21. Salivation	18
22. Watery eyes	17
23. Dyspnea	16
24. Cardiac dysrhythmia	15
25. Neck stiffness	13
26. Dysuria	13
27. Paresis	8
28. Cardiac distress	6
29. Hiccoughs	2

* Percentages in table refer to all 99 documented ciguatera cases. Percentages in text refer to symptoms observed in the 30 hospitalized cases.

Toxicological features

The data on fish ciguatoxicity, as determined by the mosquito bioassay, are shown in Table I. Also indicated are the corresponding doses of ng CTX, evaluated from the cat bioassay and the mouse bioassay results when available. We could not correlate a high level of ciguatoxicity in a fish to a severe syndrome in all its consumers.

For a more accurate analysis, the toxic fish were separated into two classes. Eighteen fish, poisonous for at least 50% of their consumers, were labelled ≥PD50 fish. Their ciguatoxicity ranged from 0.83×10^{-3} fge (inferior toxicity, IT) to 6.3×10^{-3} fge (superior toxicity, ST) with a mean toxicity (MT) evaluated to be 1.83×10^{-3} fge, from 1.1 to 8.4 AU (MT = 4.2), and from 0.26 to 1.9 ng CTX (MT = 1). Seven fish, poisonous for less than 50% of the consumers, were labelled <PD50 fish. For them, the ciguatoxicity ranged from 3.14 to 7.95×10^{-3} fge (MT = 6.02×10^{-3}), from 0.9 to 2.2 AU (MT = 1.3), and from 0.19 to 0.5 ng CTX (MT = 0.9).

Dose-effect relationship for ciguatoxin in man

In the 25 documented cases mentioned above, the average ration of fish eaten by the patients was estimated to be 0.30 ± 0.15% of their body weight (62 ± 18 kg);

TABLE III

Average and extreme doses of ciguatoxin per kg of individual weighing 62 kg and consuming 0.3% of the body weight (186 g) of poisonous fish from mouse, cat, and mosquito bioassays, expressed in animal-units and in nanograms

Dose of ingested CTX	Mouse bioassay		Cat bioassay		Mosquito bioassay		Average dose of CTX (ng) from the three bioassays
	MU	ng	CU	ng	AU	ng	
			(Batch of 18 ≥ LD50 fish)				
Maximal	2	18	4.5×10^{-2}	0.8	3.6×10^{-3}	5.7	8.2
Mean	4×10^{-1}	3.2	2×10^{-2}	0.4	1.6×10^{-3}	2.6	2.1
Minimal	6×10^{-2}	0.5	1.5×10^{-2}	0.3	0.9×10^{-3}	0.5	0.8
			(Batch of 7 < LD50 fish)				
Maximal	3×10^{-1}	2.5	3×10^{-2}	0.3	1.0×10^{-3}	1.5	1.4
Mean	8×10^{-2}	0.8	2×10^{-2}	0.1	0.6×10^{-3}	0.9	0.6
Minimal	3×10^{-2}	0.3	0.3×10^{-2}	0.03	0.5×10^{-3}	0.6	0.3

i.e., about 186 g. The doses indicated in Table III were calculated on this basis. The maximum, minimum, and mean doses of CTX toxic to man were obtained from the maximum (ST), minimum (IT), and average (MT) ciguatoxicity per gram of fish as previously defined.

The results of the mosquito bioassay were compared to those of the cat bioassay and the mouse bioassay.

(1) The PD50, expressed in ng CTX per kg of man, ranged from 0.5 to 18 (MD = 3.2) in the mouse bioassay, from 0.3 to 0.8 (MD = 0.4) in the cat bioassay, and from 1.5 to 5.7 (MD = 2.6) in the mosquito bioassay. The mean of the three bioassay values, which is considered to be the definitive PD50, ranged from 0.8 to 8.2 (MD = 2.1).

(2) The doses of CTX per kg of body weight which made at least one, but less than 50%, of the consumers sick were tentatively labelled minimum pathogenic doses (MPD). They ranged from 0.3 to 2.5 (MD = 0.8) in the mouse bioassay, from 0.3 to 3.0 (MD = 0.1) in the cat bioassay and from 0.6 to 1.5 (MD = 0.9) in the mosquito bioassay. The definitive MPD ranged from 0.3 to 0.9 (MD = 0.5).

Everyone who ate the two most toxic fish, which caused the most severe cardiovascular symptoms, required hospitalization. Considering the approximate amount of fish ingested in those cases, the PD100 for man can be estimated at 8 ng CTX/kg.

CONCLUSIONS

Ciguatoxin is one of the most potent biotoxins—via the intraperitoneal route—to mice. It also appears to be one of the most toxic natural substances to man when consumed orally. It was possible to determine a dose-effect relationship for ciguatoxin to man from the data provided when the mosquito bioassay was applied to the leftovers of 25 fish involved in 99 cases of ciguatera poisoning. Three average pathogenic doses (PD) have been defined. The minimum PD is estimated to be 0.6 ng CTX/kg

(*i.e.*, 0.7 MU/kg); the PD50 is estimated to be 2 ng CTX/kg (*i.e.*, 0.3 MU/kg); and the PD100 is estimated to be 8 ng/kg (*i.e.*, 0.9 MU/kg). These data suggest a MLD for man of approximately 20 ng CTX/kg (*i.e.*, 3 MU/kg).

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